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RADIOPROTECTION OF PRIMATES WITH 2-(1-DECYLAMINO)ETHANETHIOSULFURIC ACID IN DIMETHYL SULFOXIDE

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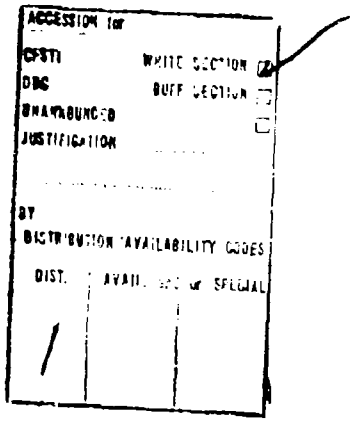

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FOREWORD

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The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences–National Research Council.

The radioprotectant, 2-(1-decylamino)ethanethiosulfuric acid, was synthesized and provided by the Walter Reed Army Institute of Research.

The authors acknowledge the assistance of the following: Nora N. Olivarez, in clinical chemistry; Yolanda L. Salmon, in hematology; and Major Harold W. Casey, in histopathology.

This report has been reviewed and is approved.

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ABSTRACT

Significant radioprotection of primates has been achieved by administering 2-(1-decylamino)ethanethiosulfuric acid, dissolved in dimethyl sulfoxide, intravenously to *Macaca mulatta*. Administered before exposure of the primates to 850 R whole-body x-radiation, this compound has resulted in 50% survival as compared with no survival of the placebo-treated primates irradiated under identical conditions. The monkeys were divided into three groups: protected and irradiated, placebo-treated and irradiated, and protected but sham-irradiated. Clinical chemistry and hematologic data were obtained preirradiation and on days 1, 3, and 7 postirradiation. Histopathologic examinations were performed on the irradiated nonsurvivors of both the protected and the placebo-treated groups and on sacrificed drug controls and radiation survivors. Hematologic data, in the form of higher WBC and platelet values, provided evidence of protection of the lymphoid system.

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I. INTRODUCTION

Several studies (1-4) have investigated the radioprotective action of 2-(1-decylamino) ethanethiosulfuric acid, hereafter referred to as WR 1607, a lipid-soluble compound synthesized by the Walter Reed Army Institute of Research. It was postulated that the lipid solubility of this drug might enhance its protective effect on the lymphoid system by preferential distribution. Storer and Yuhas (1), administering the drug in an alkaline aqueous medium to mice intraperitoneally, found the dose reduction factor to be 2.1. Further, on the basis of the amount of protection derived from a given amount of drug, WR 1607 was the most promising of 45 compounds studied. WR 1607, dissolved in Carbowax 200 and administered intraperitoneally at a dose of 10 mg./kg. 30 minutes prior to 1,200 R cobalt-60 gamma radiation, has successfully protected rats. A survival rate of 56% was achieved as compared to no survival for controls (4). No significant protection was found, however, when WR 1607 was administered in Carbowax 200, both intraperitoneally and intravenously, to *Macaca mulatta* primates before x-irradiation at a whole-body dose of 850 R (2-4).

Dimethyl sulfoxide (DMSO), noted for its exceptional solubility, its ability to penetrate through biologic tissues (5, 6) and as a radioprotectant in its own right (7-9), has been successfully used in combination with other radioprotective substances (10). It is the purpose of the present study to investigate the radioprotective effect of WR 1607 dissolved in DMSO when administered intravenously to *Macaca mulatta* before 850 R x-irradiation.

II. MATERIALS AND METHODS

Animals

With the exception of 2 irradiated and 2 sham-irradiated males in experiment 2, the primates used in the study were young adult females, *Macaca mulatta*. The weight range of the animals was 3.7 to 7.5 lb., with a mean weight of 5.4 lb. The primates were standardized by means of fecal smears and cultures, physical examinations, and cage acclimatization. They were caged individually, fed a commercial primate feed twice daily, and allowed water ad libitum.

Drug

The WR 1607 was obtained in powder form from the Walter Reed Army Institute of Research. The solubility of this compound in dimethyl sulfoxide was established as 235 mg./ml. (23° C.). The addition of small amounts of water precipitates the material, but the solution may be diluted with serum in vitro manyfold with no precipitation. Necropsy revealed no precipitation in vivo. A comparison of the infrared spectra of fresh and precipitated WR 1607 disclosed that dissolution of the substance does not result in structural changes.

The WR 1607 was dissolved in dimethyl sulfoxide immediately before injection. The primates in the preliminary experiment received 1 ml. of solution intravenously in the femoral vein by normal injection technics. The primates in the latter experiments received

2 ml. of solution per kilogram body weight by a catheter placed in the femoral vein. Placebo treatment consisted of an intravenous injection of 2 ml. DMSO per kilogram weight. All injections were given approximately 30 minutes before irradiation. One primate each received doses of 10, 15, and 20 mg./kg. of WR 1607 in the preliminary experiment. In the first experiment, 7 primates received doses of 20 mg./kg. WR 1607, 6 received 30 mg./kg. WR 1607, and 2 received placebo injections. In the second experiment, 11 animals received 26.5 mg./kg. WR 1607, 8 received placebo injections, and 3 received 26.5 mg./kg. WR 1607 but were sham-irradiated.

Irradiation

Whole-body irradiation was performed with a Maxitron 300 x-ray unit at 300 kvp, 20 ma., and standard Al-Cu-Sn filtration; half-value layer, 2 mm. Cu. The mean target distance was 135 cm., the dose rate 18.0 ± 0.5 R/min., and the total dose was 850 R. Dosimetry was performed with Victoreen ion chambers. The primates were irradiated in a cylindrical wire-mesh cage rotated at 3 r.p.m.

Clinical studies

No clinical studies were performed in the preliminary experiment. Hematologic data, consisting of measurement of leukocytes (WBC), hematocrits, and differential counts before irradiation and 72 hr. postirradiation, were obtained in experiment 1. In addition to the above hematologic data, the following data were obtained in experiment 2: platelet count, serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), lactic dehydrogenase (LDH), and blood sodium and potassium levels. Clinical chemistry and hematologic data were obtained before irradiation and on days 1, 3, and 7 postirradiation. Also, histopathology examinations were performed on 4 placebo-treated and irradiated primates, 4 protected and irradiated primates, and on 2 protected but sham-irradiated primates. These examinations were specifically made for signs of drug toxicity and protection of the lymphoid system and gastrointestinal tract.

III. RESULTS

The toxicity data are listed in table I. The LD_{50} dose is estimated to be approximately 35 mg./kg. under the experimental conditions reported. The animals died in convulsion, generally within 10 minutes of injection, and never more than 30 minutes after injection. One animal, in experiment 2, was quite weakened by the drug and appeared to be near death for about 3 days after injection and irradiation; however, it survived until the 11th day postirradiation. No placebo-treated animals exhibited signs of toxic effects. This is in agreement with Feinman et al. (11), who reported no deaths among *Macaca mulatta* which received daily doses of 4 gm./kg. DMSO intravenously 5 days a week for 14 consecutive weeks.

The survival data are presented in table II and the survival curves in figures 1 and 2. It will be noted from these figures that the mortality patterns for the protected and the placebo-treated primates are essentially the same for the initial two weeks after irradiation. All deaths occurred between the 7th and 17th days postirradiation in both groups. However, 50% of the animals which received 15 mg./kg. or higher doses of WR 1607 survived whereas none of the placebo-treated primates survived. For the purpose of preparing the mean survival times, the survivors

TABLE I

Toxicity results

Dose (mg./kg.)	Deaths/animals*
0 (Placebo)	0/10
10	0/1
15	0/1
20	0/10
26.5	1/14
30	1/7
40	2/3

*Number of animals differs from number shown in table II because some animals were used for drug toxicity studies only.

TABLE II

Survival data

Dose (mg./kg.)	Survival ratio	Percent survival	Mean survival time in days (including survivors)	Mean survival time in days (excluding survivors)
0	0/10	0	13.3	13.3
10	0/1	0	19.0	19.0
15	1/1	100	80.0	—
20	5/8	62.5	23.3	12.0
26.5	3/10	30.0	17.9	12.7
30	3/5	60.0	21.8	9.5

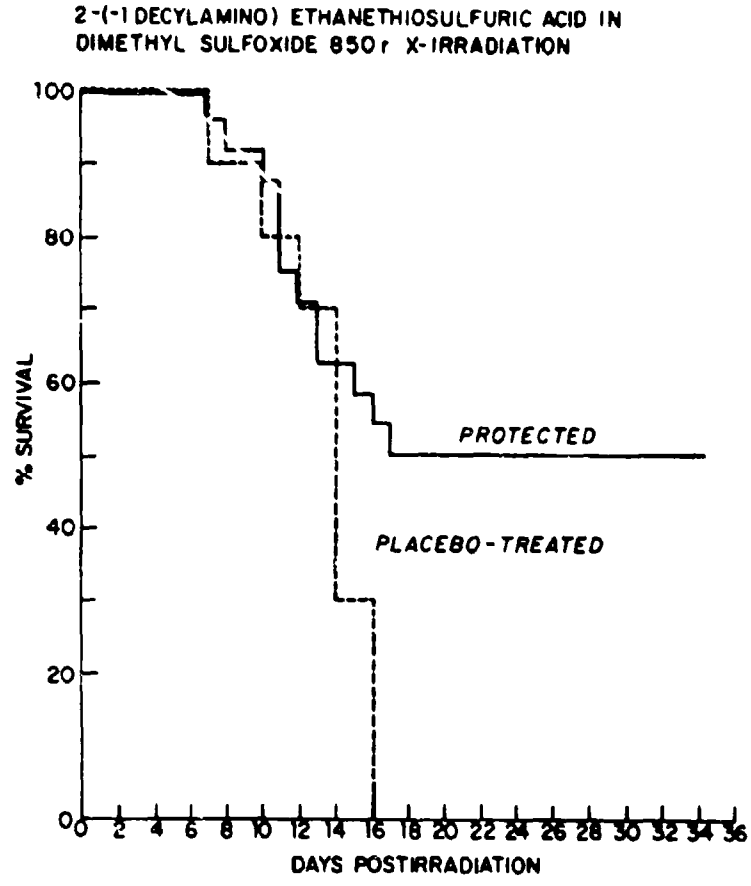

FIGURE 1

Protected primate survival versus unprotected primate survival after 850 R x-irradiation.

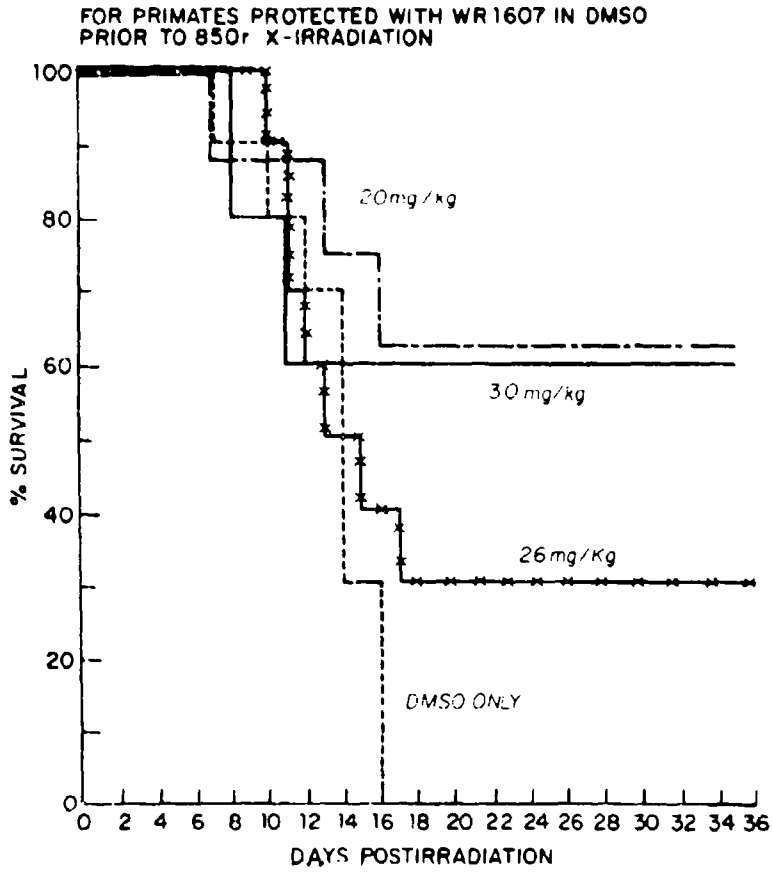

FIGURE 2

Protected primate survival at three dose levels versus unprotected primate survival after 850 R x-irradiation.

were arbitrarily assigned a survival time of 30 days. Survivors have now been observed for periods of 3 to 5 months postirradiation and, excluding primates sacrificed for additional studies, no deaths have occurred after the initial 17 days.

The hematology means for e.:periment 1 are presented in table III. The chemistry and hematology means for the second experiment are presented in tables IV and V, and in figures 3 to 5. The mean WBC values decreased postirradiation for each group of primates, but a comparison of the percent decrease offers evidence of protection of the lymphoid system. In the first experiment, the percent change from the preirradiation values to the day 3 postirradiation values was 56.5% for the placebo-treated primates, 54.9% for the animals given 20 mg./kg., and 53% for the animals given 30 mg./kg. The differences in the percent change do not appear significant, but the same pattern is evident in the second experiment—a 72.4% decrease for the placebo-treated animals as compared to a 46.7% decrease for the protected primates.

TABLE III

Hematology means, experiment 1

Group	WBC (cells/mm.^{3})*		Hematocrit (%)		Neutrophils (%)		Lymphocyt	(%)
	Pre-†	Post-‡	Pre-	Post-	Pre-	Post-	Pre-	-t-
Placebo-treated	5.2	2.3	40.5	33.5	25.5	66.5	73.0	1.5
20 mg./kg.	10.1	4.6	38.5	32.8	18.2	80.7	79.2	3
30 mg./kg.	10.9	5.2	39.8	31.0	22.6	83.0	73.2	9

*×10^3.

†Preirradiation.

‡Day 3 postirradiation.

TABLE IV

Chemistry means, experiment 2

Parameter	Placebo-treated and irradiated				Protected and irradiated				Protected and sham-ir			ated
	Pre-*	Day 1†	Day 3†	Day 7†	Pre-	Day 1	Day 3	Day 7	Pre-	Day 1	Day 3	Day 7
SGOT (units/ml.)	31.8	104	58.6	38.7	46.1	23b	68.4	40.4	47.0	102	66.3	43.3
SGPT (units/ml.)	30.2	51.2	34.1	33.4	29.1	55.6	49.4	20.1	33.7	42.3	31.3	19.7
LDH (units/ml.)	544	2,910	1,090	655	556	4,070	2,570	827	547	2,510	1,140	8 15
Na (mEq./ml.)	154	148	153	152	152	150	148	144	153	160	148	154
K (mEq./ml.)	4.9	4.6	4.5	4.4	5.4	4.8	5.5	4.1	5.0	4.7	5.0	4.6

*Preirradiation.

†Postirradiation.

TABLE V

Hematology means, experiment 2

Parameter	Placebo-treated and irradiated				Protected and irradiated				Protected and sham-ir			ated
	Pre-*	Day 1†	Day 3†	Day 7†	Pre-	Day 1	Day 3	Day 7	Pre-	Day 1	Day 3	ay 7
WBC (cells/mm.^{3})‡	12.8	11.0	3.5	0.4	11.0	17.4	5.9	1.4	10.4	14.6	9.4	7.8
Platelet (cells/mm.^{3})‡	355.0	196.8	251.8	252.8	308.1	326.5	328.8	167.1	353.7	68.3	253.0	46.3
Hematocrit (%)	39.9	35.1	30.6	28.9	38.8	33.3	30.4	26.2	39.7	33.7	30.3	30.9
Neutrophil (%)	21.4	84.9	78.4	36.3	22.9	90.3	91.7	41.0	15.7	51.3	52.3	36.3
Lymphocyte (%)	76.9	13.4	17.8	63.7	71.5	8.7	8.1	58.8	81.7	46.0	46.7	3

*Preirradiation.

†Postirradiation.

‡×10^3.

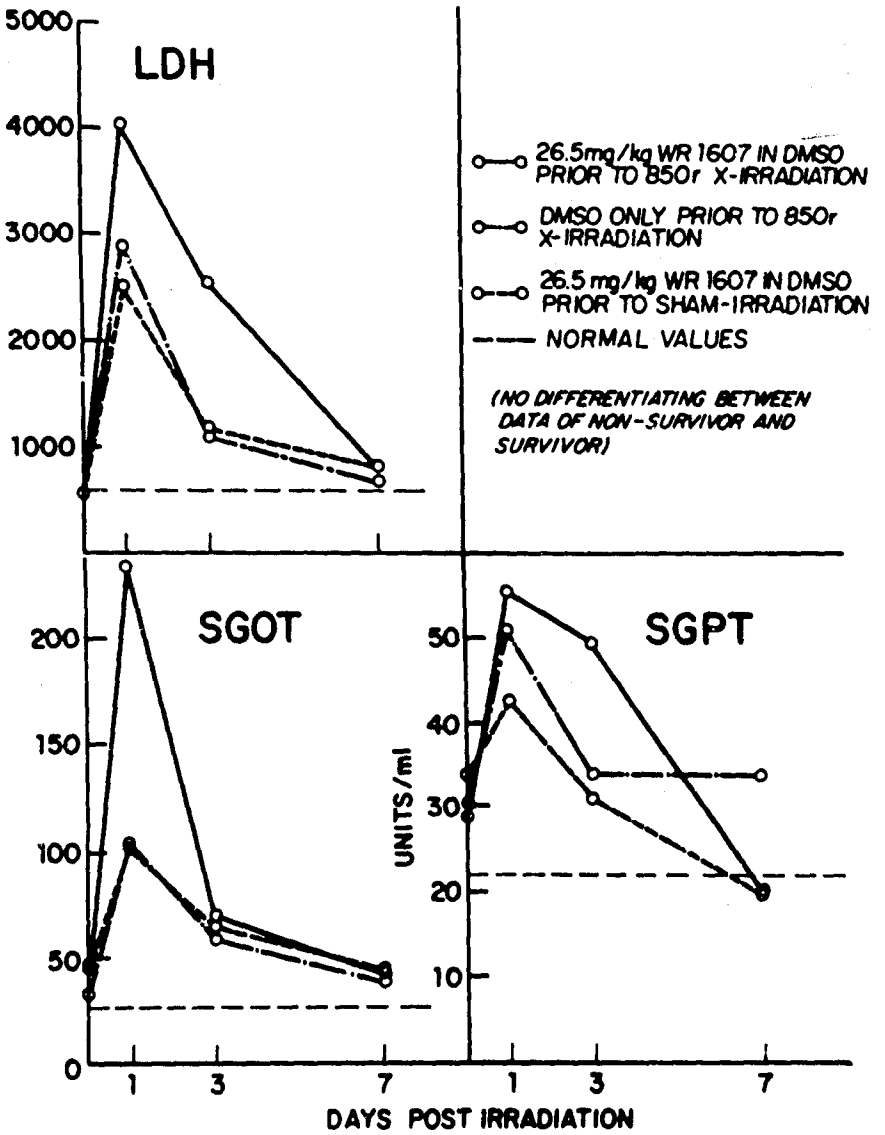

FIGURE 3

Means of three blood chemistry parameters as seen in three different experimental groups. Normal values are as listed in reference 12.

The blood chemistry response (fig. 3) was similar for the placebo-treated irradiates and for the chemoprotected nonirradiates, but the SGOT, SGPT, and LDH values for the protected irradiates exhibited an initial postirradiation increase well in excess of the other two groups, thus suggesting a possible potentiative effect between the compound and the radiation. This possible potentiation was also indicated in the neutrophil-lymphocyte ratio means. The WBC portion of the hematologic response (fig. 4) suggested a degree of protection to the lymphoid system in that the means for the protected primates were greater than those of the placebo-treated primates at days 3 and 7 postirradiation.

Table VI compares the day 7 postirradiation platelet and WBC means for the animals of the second experiment with data obtained by

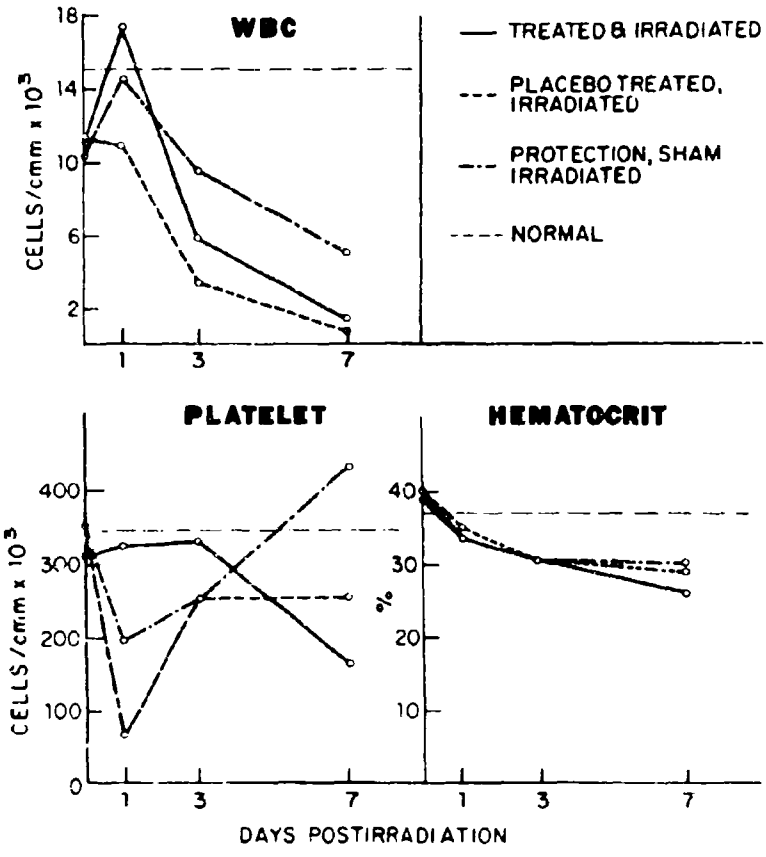

FIGURE 4

Means of three hematology parameters as seen in three different experimental groups. Normal values are as listed in reference 13.

other investigators. One group was treated with WR 1607 dissolved in Carbowax 200 (3); another, with a mixture of 5-hydroxytryptamine (5-HT), 5-methoxytryptamine (5-MT), and sodium hexobarbital (4); and the third group was not treated (4). All were irradiated under conditions identical to those of the present study. A comparison of the WBC means for the animals protected with WR 1607 in DMSO against the means for the other four groups provides additional evidence of protection of the lymphoid system (1.40×10^3 compared to 0.44 to 0.53×10^3 cells per cubic millimeter). The platelet means also indicate protection, but this appears to be, at least in part, attributable to the dimethyl sulfoxide. At the dose applied in this study, however, the DMSO, by itself, provided insufficient protection to enable the animals to survive.

Blood chemistry and hematologic data were obtained for 2 protected irradiates of the second experiment on day 37 postirradiation. These data are presented in table VII. The platelets, eosinophils, and SGOT values were high for each animal as compared to normal values (12), and 1 animal had an abnormally high WBC count. The other values were essentially normal.

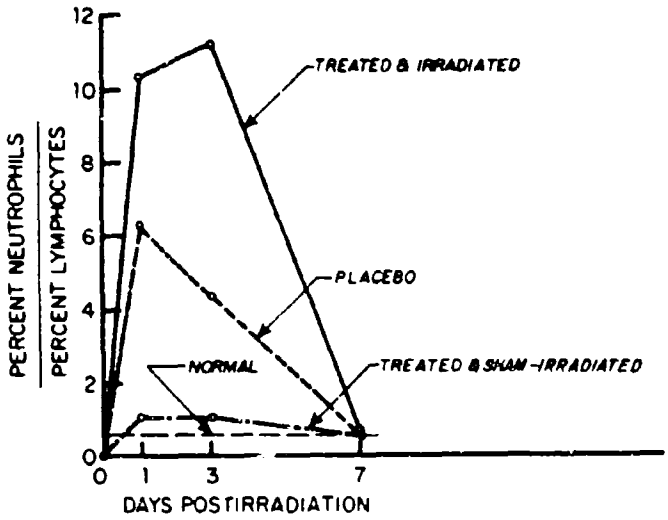

FIGURE 5

Means of neutrophil-lymphocyte ratio in three different experimental groups.

TABLE VI

Platelet and WBC values of protected and nonprotected primates

Protectant	Survival ratio	Number of animals tested	Platelets* (cells/mm.^{3})†	WBC* (cells/mm.^{3})†
WR 1607 in DMSO	12/24	10	167.0	1.40
WR 1607 in Carbowax 200	2/20	16	52.8	0.51
5-HT, 5-MT, and sodium hexobarbital	1/21	20	73.0	0.53
DMSO	0/10	7	253.0	0.44
None	0/5	5	81.4	0.51

*Seven days after irradiation by 850 R gamma.
†$\times 10^3$.

Histopathologic examinations failed to differentiate between the radiation deaths incurred by placebo-treated and protected primates. The nonsurvivors of both groups exhibited the typical signs of radiation damage—bone marrow aplasia, lymphoid hypoplasia, widespread hemorrhages, and bacteremia. Also, the examinations revealed no signs of drug toxicity in either the protected primates, or those which received DMSO only. The 2 radiation survivors, from which day-37 blood samples were obtained, were sacrificed on the 37th day postirradiation. Histopathology studies revealed slight signs of radiation damage but ample evidence of recovery. The viscera were essentially normal;

TABLE VII

Day 37 postirradiation chemistry and hematology means

Primate	SGOT (units/ml.)	SGPT (units/ml.)	LDH (units/ml.)	Na (mEq./ml.)	K (mEq./ml.)	WBC* (cells/mm.^{3})	Neutrophils (%)	Lympho-cytes (%)	Eosinophils (%)	Platelet* (cells/mm.^{3})	Hematocrit (%)
39F	68	49	605	149	4.3	19.6	21	39	40	501	38
43F	60	71	875	156	5.3	9.4	21	47	32	632	37

*$\times 10^3$.

there was mild lymphoid hypoplasia accompanied by evidence of regeneration; and the bone marrow was extremely hyperplastic. Essentially 100% of the marrow spaces were filled with cells, the predominant cell being the granulocytic type.

IV. DISCUSSION

Previous radioprotection studies utilizing WR 1607 have been severely restricted by the insolubility of this compound in water. The use of dimethyl sulfoxide, which combines low toxicity with suitable solubility characteristics, greatly increases the applicability of this drug. The lack of placebo-treated survivors in the current study is evidence that, at the doses applied, DMSO alone is not an adequate protectant. Furthermore, infrared spectra have provided evidence that WR 1607 and DMSO do not chemically combine. Thus, the radioprotection observed in these studies, in contrast to the absence of protection when WR 1607 is dissolved in Carbowax 200, is attributed primarily to the fact that the solvent distributes the radioprotectant in the organism more efficiently. Indeed, studies have shown dimethyl sulfoxide to be an efficient carrier for numerous drugs (5, 6).

The present study was felt to be particularly successful since protection (62.5% survival) was achieved at a drug dose sufficiently low that no toxicity deaths occurred. In addition, the higher WBC values of the protected animals in comparison to the corresponding values for the placebo-treated primates, and the higher WBC and platelet values of the protected animals as compared to those of nontreated primates and primates treated with other radioprotectants (2-4), provided physiologic evidence of protection. Histopathology examinations, however, failed to confirm the observed protection.

Since only a small number of animals was studied, the difference in percent survival between the various groups of primates (fig. 2) is not significant. The lower survival rate in the final experiment may be attributed to the trauma induced by the increased samplings of this group. The trauma of drawing 6 ml. of blood from an animal at a time when the WBC count has decreased to approximately 10% of the normal may have been sufficient to weaken a few borderline cases to the point where they could no longer recover. It may be possible to further enhance the protective action of WR 1607 by eliminating postirradiation blood samplings, and by increasing the volume of DMSO, varying the interval between injection and irradiation, and using the drug in conjunction with other radioprotectants. One radioprotectant which might be successfully used in conjunction with WR 1607 is S-2-(3-aminopropylamino)ethylphosphorothioic acid hydrate, a water-soluble drug synthesized by the Walter Reed Army Institute of Research. Studies have shown that *Macaca mulatta* can tolerate a combination of WR 1607 (20 mg./kg.) and the water-soluble drug (250 mg./kg.)—dosages which, individually, are capable of providing sufficient protection to achieve 60% survival of the animals after x-irradiation by 850 R (14).

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13. ABSTRACT

Significant radioprotection of primates has been achieved by administering 2-(1-decylamino) ethanethiosulfuric acid, dissolved in dimethyl sulfoxide, intravenously to Macaca mulatta. Administered prior to whole body X-irradiation by a dose of 850 roentgens, this compound has resulted in fifty per cent survival, as compared to zero per cent survival of the placebo treated primates irradiated under identical conditions. Clinical chemistry and hematologic data were obtained preirradiation and on days one, three, and seven postirradiation for three groups of primates; protected and irradiated, placebo treated and irradiated, and protected but sham-irradiated. Histopathology examinations were performed on the radiation non-survivors of both the protected and placebo treated animals, and on sacrificed drug controls and radiation survivors. Hematologic data, in the form of higher WBC and platelet values, provided evidence of protection of the lymphoid system.

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